

Survey of Commercial Milk Samples for Aflatoxin M

Abstract

A survey of milk samples was undertaken to determine if commercially available milks in the United States were contaminated with aflatoxin M. Among the samples were bottled whole milks, dried skimmilks, and canned evaporated milks. Bulk whole milk samples and some samples collected directly from the farm were also surveyed. No aflatoxin M was detected in any of the milk samples.

Introduction

Allcroft and Carnaghan (1, 2) and deLongh et al. (3) reported that cows fed groundnut meal containing aflatoxin B₁ excreted into the milk a "toxic factor" that was unlike any of the other aflatoxins. Holzapfel et al. (4) showed that this toxic factor or milk toxin contained two fluorescent components subsequently designated M₁ and M₂. Their structures were then determined by Holzapfel et al. (4) and Masri et al. (5) and were found to be hydroxylated derivatives of the aflatoxins B₁ and B₂.

The amount of M (both M₁ and M₂) in milk (8) is proportional to the intake of the B₁ concentrate and represents significantly less than 5% of the ingested dose of B₁. Aflatoxin M is also in urine and feces of the bovine in comparable amounts (8). Besides metabolic conversion of B₁ by the cow, M also exists naturally as a metabolic product of organisms belonging to the genus *Aspergillus* (4).

Aflatoxin B₁ is carcinogenic (10) in many experimental animals. Similar laboratory experiments suggest that aflatoxin M may also be carcinogenic, although this has not been proven conclusively. Hydroxylation of B₁ to M₁ has little effect on its toxicity (4). There is no direct evidence for the susceptibility of man to aflatoxins. Work with monkeys showed that man could be susceptible. If in milk, the real problem may be the cumulative effect of repeated exposure to very small doses over a long period.

Because of the danger of M occurring in the milk and knowing that it is almost as toxic as B₁ and may be carcinogenic, milk surveys have been undertaken. Allcroft and Carnaghan (2) failed to detect any contaminated milk from pooled milk samples in Great Britain. However, Purchase et al. (9), in South Africa, confirmed aflatoxin M in 1 out of 21 commercial milk

samples from cows being fed groundnut meal.

The present survey was undertaken to determine if commercially available milks in the United States are contaminated.

Experimental Procedure

The method for detection of aflatoxin M was, with slight modifications, the method described by Masri et al. (6, 7). For fluid milk, 75 ml of methanol were added to 50 ml of the milk. The mixture was allowed to stand for 10 minutes and then centrifuged at approximately 15,000 rpm for 10 minutes. One hundred milliliters of the supernatant were decanted into a graduated cylinder and extracted with 3 × 50 ml of chloroform in a separatory funnel. The chloroform extract containing the aflatoxin was washed with 3 × 75 ml of 50% water in methanol and once with 75 ml of water. The chloroform extract was then evaporated to dryness on a steam bath and the residue taken up in 500 μ liters of chloroform. Five μ liters of this chloroform solution were spotted onto an Absorbosil-1 (Applied Science Labs., State College, Pa.)¹ thin-layer plate and eluted with 15% acetone in chloroform in a nonequilibrated chromatographic tank.

The procedure for dry milk consisted of defatting 10 g of the dried milk with petroleum ether, filtering and air drying the sample, and extracting with 100 ml of 20% water in methanol in a blender. The extract was filtered and the filtrate extracted with 3 × 50 ml of chloroform. The rest of the procedure followed that of the fluid milk.

The M standard was obtained from the freeze-dried milk of a cow that had been on high levels of a B₁ concentrate. It contained 100 ppb of the aflatoxin M based on the weight of the dry sample. Once every two weeks a new standard was prepared as a precautionary measure. Using the reconstituted standard, the sensitivity was visually less than 1 ppb on a fluid milk basis.

Polyethylene bottles (250-ml capacity) containing 2 ml of a preservative, Combiotic, (Pfizer Chem. Co., New York, N. Y.) were used for mailing samples, which were sent by regular mail unrefrigerated in mailing tubes.

¹ Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

Results and Discussion

First to be examined were bottled whole milk, dried skim milk, and canned evaporated milk samples from markets in Washington, D.C. and Philadelphia, Pa. About 20 different brands of milk were analyzed and no aflatoxin M was detected. Dried whole milk samples from various seasons prepared at our laboratory over a 3-year period were screened; again no M was found. The scope was then broadened and through the cooperation of the Dairy Division of the Consumer and Marketing Service, USDA, we obtained samples through its Market Administrators from Phoenix, Ariz.; Chicago, Ill.; Hartford, Conn.; San Antonio, Tex.; Philadelphia, Pa.; Milwaukee, Wis.; Boston, Mass.; Seattle, Wash.; Rock Island, Ill.; Albuquerque, N. M.; and Fort Lauderdale, Fla. From each area 20 samples were collected from tank trucks where practicable and all samples were negative. Since dilution due to pooling could lower small amounts of contamination below the detectable limit, arrangements were made to obtain single farm samples. This was done through cooperation with the Dairy Husbandry Division of the Agricultural Research Service, USDA, in Beltsville, Maryland, and the Maryland Cooperative Milk Producers, Inc., in Baltimore, Maryland. Samples were collected from farms known to be feeding moldy corn silage and from 150 more locations in the Maryland area. No aflatoxin was found. Samples suspected of possible contamination in California were collected through cooperation with the Bureau of Dairy Science, California Department of Agriculture, but the results were all negative.

The results indicate that there is no cause for general concern about aflatoxin contamination in milk in the United States. This does not mean that contamination might not occur in isolated cases, since it is known that feeding contaminated feeds, particularly peanut meal and possibly cottonseed meal, can lead to aflatoxin M in the milk. We are continuing to explore areas and conditions which might lead to aflatoxin-contaminated milk.

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C. R. BREWINGTON and **J. L. WEIHRAUCH**, Dairy Products Laboratory, Eastern Utilization Research and Development Division, ARS, USDA, Washington, D.C. 20250 and **C. L. OGG**, Plant Products Laboratory, Eastern Utilization Research and Development Division, ARS, USDA, Philadelphia, Pennsylvania 19118

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